In response to the Examiner's objections to the claims, applicants have amended claims 1-12, cancelled claims 13-18, and added new claims 19-22. Claims 19-22 incorporate the subject matter previously claimed in "use" claims 15-18, and therefore respond to the Examiner's objection under 35 USC §112, second paragraph, and rejection under 35 USC §101.

With regard to claims 1-12, applicants have addressed the Examiner's objections by deleting "preferably" where present in the claims. Applicants have also clarified the claims to satisfy the Examiner's other objections.

The Examiner inquired as to how the administration of the composition of claim 1 results in the sustained release of endothelial prostacyclin, as recited in claims 5 and 18 (new claim 22).

Applicants submit the following response.

In the specification bridging pages 12 and 13, it is stated that polydeoxyribonucloetides according to the invention have an antithrombotic, anti-ischa emic, cytoprotective, anti-inflammatory and anti-atherosclerotic activity. These activities are obtained by the local release of endothelial prostacylcin in the blood flow in therapeutically effective amounts.

The activity of the polydeoxyribonucloetides in the listed pathologies is preferable to the induced local release of prostacyclin.

As stated in the specification at lines 5-9 on page 13, the liposome complex disclosed in present invention affords a sustained release of endothelial prostacyclin.

Applicants have also demonstrated in Example 6, page 22 of the specification and in Example 4, page 17 of the specification, that both antithrombotic and anti-inflammatory activities, which as indicated above are among the pharmacological effects caused by prostacyclin release in the blood flow, are maintained in vivo even after conditioning the liposome solution at 25 °C for 30 days. See Table III on page 29 and Table I on page 27 of the specification.

In light of this explanation, applicants respectfully submit that the claims are clear.

Claims 1-18 are rejected under 35 USC §103(a) as being unpatentable over Applicants' statement of prior art in combination with Litzinger (1996), alone or in combination with Maccarone (1992) and Eastman (1997), individually or in combination.

The Examiner indicates that Applicants note on pages 3-7 of the specification that the polydeoxyribonucleotides of the invention are known for their function. The Examiner relies on Litzinger for disclosing oligonucleotides having the inability to efficiently traverse through cellular membranes, and therefore should be complexed with cationic liposomes (the Examiner refers to the Abstract and page 140). The Examiner relies on Maccarone as teaching that DNA, when complexed with cationic liposomes, are able to transfect protoplasts (the Examiner refers to the Abstract). The Examiner relies on Eastman for teaching that complexes prepared by the addition of cationic liposomes and DNA have efficient transfection ability (the Examiner refers to the Abstract and the "materials and methods" section).

The Examiner takes the position that the use of cationic liposomes for the delivery of art-known polynucleotides would have been obvious to one of ordinary skill in the art in view of the teachings of Litzinger, Maccarone and Eastman.

This rejection is respectfully traversed.

It is known that liposome complexes with polynucleotides or oligonucleotides have the property to remarkably increase the pharmacological properties of the polymers (see page 3 of the specification, lines 11-14).

The preparation of liposome complexes containing polydeoxyribonucleotides of molecular weight 16,000 obtained by depolymerization of nucleic acids is also well known in the prior art. (See page 3, lines 5-95 of the specification.)

However, Applicants have found that these complexes are unstable and besides, produce toxic effects in experimental animals.

Lack of stability means that the polydeoxyribonucleotides obtained by depolymerization of nucleic acids in the complexes results in a very rapid reduction of the pharmacological activities (see page 3, lines 15-19 of the specification) when formulated in compositions for parenteral administration.

See the Tables in the specification which show the decrease with time of the following pharmacological activities in vivo (rats):

Anti-inflammatory activity: Table I at page 27, the row corresponding to group No. 3. Decrease in activity over 30 days: 78.8%

Anti-hypertensive activity: Table II at page 28, the row corresponding to group No. 4. Decrease in activity over 30 days: 70%

Anti-thrombotic activity: Table III at page 29, the row corresponding to group No. 4. Decrease in activity over 30 days: 73.3%

The above shows that the liposome complexes with polydeoxyribonucleotides of the prior art could not be effectively used in therapy.

Besides this symptoms of toxic effects were noted in the animals treated with the above formulations in the anti-inflammatory activity test (see page 19, Example 4, lines 19-20) and in the anti-hypertensive activity test (see page 22, Example 5, lines 10-11).

Observations in the test for anti-thrombotic activity could not be made since, as seen in Example 5 on page 22 of the specification, the animals did undergo surgery and were kept under anesthesia.

In conclusion, the technical problem to be solved was in providing stable liposome complexes of polydeoxyribonucleotides obtained by depolymerization of nucleic acids, for use according to claim 1, i.e. for delivering polydeoxyribonucleotides by parenteral administration.

The applicant has found that this technical problem can be solved by preparing liposome complexes of polydeoxyribonucleotides obtained by depolymerizing nucleic acids wherein the polymers are attached to the external surface of the liposome vesicle.

The polydeoxyribonucleotides in the claimed liposome complexes display an activity equal or comparable to that of the liposomes of the prior art mentioned in the application. However, in contrast to the prior art, the activity of the formulation of the new complexes is maintained for a long time (page 4, lines 19-22) and toxic effects are absent.

This has been demonstrated by the Applicant for: anti-inflammatory activity in the group of rats No. 2 of Table I; antihypertensive activity in group No. 3 of Table II; antithrombotic activity in group No. 3 of Table III.

All groups of rats were treated parenterally with the liposome complex of polydeoxyribonucleotides according to the invention. Note that no occurrence of toxic effects was reported in the pharmacological tests performed on non-anesthetized animals. See the conclusions of Example 4 on page 19 and of Example 5 on page 22.

The liposome complexes of the invention are much more stable and devoid of toxic effects, thus they are suitable for use in therapy.

Turning now to the Examiner's rejection, Applicants note the following. Litzinger has been mentioned in the application as a reference for the process of preparation of cationic liposomes with polydeoxyribonucleotides used in the present invention.

Applicants have noted that at page 140 of this paper (upper part of the left column) it is stated that cationic liposomes have the inability to efficiently traverse through cellular membranes, and that inconvenience can be overcome by using cationic liposomes.

However this disclosure does not help the artisan to distinguish between the stability of liposome complexes with polydeoxyribonucleotides, wherein the deoxyribonucleic acids are located inside the liposome, rather than those wherein said nucleic acids are attached on the outside surface of the liposome. In other words, there are no suggestions in Litzinger pointing to liposome/DNA complexes that could be stable over time in vehicles used for intravenous formulations.

Besides this, no information are given on the toxicity of the complexes. In conclusion the paper of Litzinger does not seem relevant to the present claims.

Maccarone teaches that in order to overcome the permeability barrier imposed by the cell membrane, and to facilitate the introduction of exogenous genes into eukaryotic cells (transfections), DNA plasmids must be complexed with polycationic liposomes. See page 1417 of Maccarone.

It is noted that Maccarone uses plasmids with a molecular weight from 4.2 Kb to 12.8 Kb. In fact, since one base unit, i.e. the average nucleotide mass, corresponds to a molecular weight of about 330, it can be concluded that the above molecular weighs are well above 500,000 Da, i.e. far away from the limits recited in claim 1 of the present application.

It is further remarked that the liposome complexes are toxic when tested in vitro. Fig. 2 at bottom of page 1420 of Maccarone shows in fact that cell viability drastically decreases by increasing the liposome concentration.

On top of page 1421 of Maccarone it is also stated that longer incubations of the liposomes with the protoplasts, up to 60 minutes, reduced cell viability from 80% (determined at 30 minutes) to 60% (60 minutes), thus indicating cytotoxicity.

Since the liposomes of the present claims are for in vivo administration, the artisan could not have found in Maccarone a suggestion for solving the technical problem of the present invention.

Eastman discloses aerosol formulations of cationic lipid DNA complexes giving a high level of gene expression in mouse lung.

The DNA used in these complexes has an unspecified molecular weight. However, since the DNA is defined as a plasmid DNA (see line 8 of the Abstract at page 765), it can be concluded that it has a very high molecular weight.

In the literature of the field, it is known that plasmids have a length of at least 2,000 bases. See the enclosed page 695 from the book "Biochemistry - 3rd Ed. 1975".

Since the molecular weight of the average nucleotide is about 330 (see Eastman, lines 10-11, right column of page 766), then a length of 2,000 bases corresponds to a molecular weight of 660,000 Da, far away from the limits recited in the present case.

From Eastman it is also drawn that the administration in vivo of these complexes in aerosol formulations causes toxic effects.

At page 766, in the column on the left, prior to the paragraph "Materials and methods", it is stated that mice administered intranasally with the complexes exhibited much greater levels of acute inflammation than mice exposed to a cationic lipid plasmid DNA aerosol.

The same is repeated at page 773, the column on the right wherein it is stated "... it may be expected that the acute toxicity observed when cationic lipid: pDNA are instilled may be moderated by distributing the complex throughout the lung ..."(emphasis added and citation omitted).

In conclusion, the aerosol formulations of Eastman, although claimed to be safer than the ones of the same liposome complexes administered by instillation, appears to still give toxic effects.

However, taking into account that in the present case, the liposome formulations are for parenteral administration, Applicants submit that Eastman fails to suggest liposome complexes for use in therapy by parenteral administration, since the physician would expect that compounds found toxic by nasal instillation would also be toxic when administered parenterally, i.e., directly into the blood stream. The teachings of Eastman would not help the artisan to identify those liposome complexes that could be safely administered by intravenous route.

It is also noted in the right hand column on page 768 of Eastman (before the paragraph "Optimization of the aerosol formulation in vitro"), that after 40 minutes, a quantity less than 10% of the plasmid DNA complexed to cationic lipid has been degraded.

The above statement makes reference to Fig. 1C at the bottom of the page. As it is explained in the footnotes of Fig. 1, the figures shown (A, B and C) represent the kinetics of DNA degradation of pDNA in the aerosol solution.

It is remarked that in the case of an aqueous solution containing the liposome complex of the present invention, after 30 days the titer of the polydeoxyribonucleotides decreases on the average of 12%.

That means that the liposome complex of the invention degrades to nearly the same extent as the one of Eastman, but in a period about a thousand times longer than that reported for the plasmid-liposome complex of the reference.

The percentage of 12% has been calculated on the basis of the decrease in activity in the experiments of anti-inflammatory, anti-hypotensive and anti-thrombotic activities, given respectively at page 27, Table I, 1st row (-12.7%); page 28, Table II, 1st row (-9.3%); page 29, Table III, 1st row (-12.9%).

This percentage decrease of the polydeoxyribonucleotide titre, calculated by averaging between three different pharmacological assays, must be taken as highly reliable.

Thus, Eastman does not seem to be a suitable reference for preparing liposome complexes for use in therapy for intravenous administration, that should be both non-toxic and stable over time.

For the above-noted reasons, Applicants respectfully submit that the combination of the cited references would not render obvious the present invention. Applicants request

that the rejection be withdrawn.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,
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